

Sucrose movement and metabolism in small Hass avocado fruit

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ABSTRACT

The transport (or accumulation) of photosynthate, plant hormones, signalling molecules and small proteins/nucleic acids from the source to the sink, within each individual cell plays a vital role in the control of tissue and cellular metabolic activity. Thus alterations in the movement of these molecules and the route by which they move can be expected to impact on tissue metabolism, growth and development. Ultimately these changes in tissue physiology will play an important role in determining fruit size.

INTRODUCTION

Stress can be defined as any factor that places a demand on the plant's energy, such as 'persistent sub optimal environmental conditions' (Bohnert & Sheveleva, 1998). Perception and signalling of this stress will lead to changes in biochemical pathways that act to alter plant physiological reactions and so protect the plant from this stress, a process termed 'a stress response'.

The plant cell can be considered to have a delicate balance of molecules and ions, both synthesized by the plant or taken up from the environment. This balance of molecules and ions is kept within a narrow range and so maintains a relatively stable physiological environment/equilibrium within the cellular organelles/compartments, the cell, the tissue and the plant and the environment, by a process that is collectively called homeostasis (Greek: *homos*, similar; *stasis*, standing). Transition through developmental stages; programmed cell death and senescence; external stimuli, such as osmotic, pathogenic, nutritive and physical stress; predation; and natural or man-made toxic compounds will cause a change in the concentration of some of these molecules/ions and via a cascade of events that will further change the concentrations of these molecules and ions, cause changes in gene expression manifested as changes in enzyme activity, metabolism, hormone fluxes/activity and ultimately overall tissue physiology. Thus it can be seen that nearly all processes in the fruit, for instance, are intrinsically linked in a complicated web, such that changes in any factor can be expected to cause changes in another. To date it is understood that hormones (Grill & Himmelbach, 1998)(such as ABA, CK and auxin); sugars (Smeekens, 1998), especially glucose (Sheen *et al.* 1999), and the seven carbon sugar manno-heptulose found in high concentrations in avocado which acts as a hexokinase inhibitor (Pego *et al.* 1999); and ions (such as calcium (Ca^{2+}) and

phosphate (PO_4^{3-}) play a vital role in mediating endogenous developmental programmes, perceiving and transcribing extra cellular signals and so regulating and optimising plant growth.

Thus it is of particular interest that the abscisic acid (ABA) content of Hass fruit was shown to be negatively correlated to fruit growth and that manipulation of the endogenous cytokinin (CK)/ABA ratio appeared to reduce cell-to-cell transport (Moore Gordon *et al.* 1998). The reasons for this interest are three fold. Firstly, these results suggest a clear link between the plant hormone ABA, symplastic transport and fruit growth. Secondly it is well understood that symplastic transport is, during normal linear fruit growth, the primary route of solute transport. And thirdly, sugar transport and metabolism in the sink is very important in relation to fruit growth as it affects sink strength, sink turgor, provides the basic building blocks for many structural and messenger molecules (including plant hormones and DNA/RNA)(Yu, 1999) and provides the substrates for metabolism. Furthermore, many plant genes are controlled by sugars, including those involved in photosynthesis, storage protein accumulation, and starch, lipid and nitrogen metabolism (Pego *et al.* 1999). Thus it is clear that any alteration in sugar supply and availability will impact on hormone signalling, gene expression and subsequent sugar import, metabolism and signalling and so affect overall fruit physiology and growth. No single growth regulator (hormonal or otherwise) can control fruit morphogenesis (Trewavas, 1983) but rather growth substances (including hormones and sugars) exert multiple controls on development through changes in concentration and changes in tissue responsiveness during fruit development (Trewavas, 1982, 1991; Firn, 1986). Thus, it must be understood that developmental programmes and fruit morphogenesis are not merely a consequence of limited resources or altered hormone levels, but a consequence of a complex spatial and temporal interaction between these two factors and many more.

Over the last few years the small fruit phenotype of Hass has generated a good deal of interest and has been the subject of much research, with success in some aspects of its physiology. Research by Moore-Gordon and coworkers (1996; 1997) suggested that the occurrence of the small fruit phenotype is aggravated by stress, particularly water stress, and could be ameliorated by mulching. However at that stage the Hass small fruit problem was still ill-defined and poorly understood (Cowan *et al.* 1997), and although there is now a much clearer and is the primary form (Sheen *et al.* 1999; Sturm & Tang, 1999) in which assimilated carbon is transported. In some species sugar alcohols (e.g. sorbitol, mannitol and in avocado this may include the abundant (Liu *et al.* 1996) sugar alcohol perseitol) and sugars of the raffinose series (e.g. raffinose and stachyose) are the principle forms of transported sugars, but where these predominate sucrose is always present. Sucrose serves as a compound for the long distance transport of metabolites; to drive osmotic solute movement; activate/repress many genes (Koch, 1996); and via the sucrose sensing pathway can modulate transport activity and assimilate partitioning (Chiou & Bush, 1998). It is understood that the picture of the processes associated with the development and occurrence of small fruit (see Cowan *et al.* 1998; Cripps *et al.* 1999) the specific stimuli which result in the initiation of the small fruit has not yet been elucidated.

The objective of this research was to thus try and see if there were differences in solute

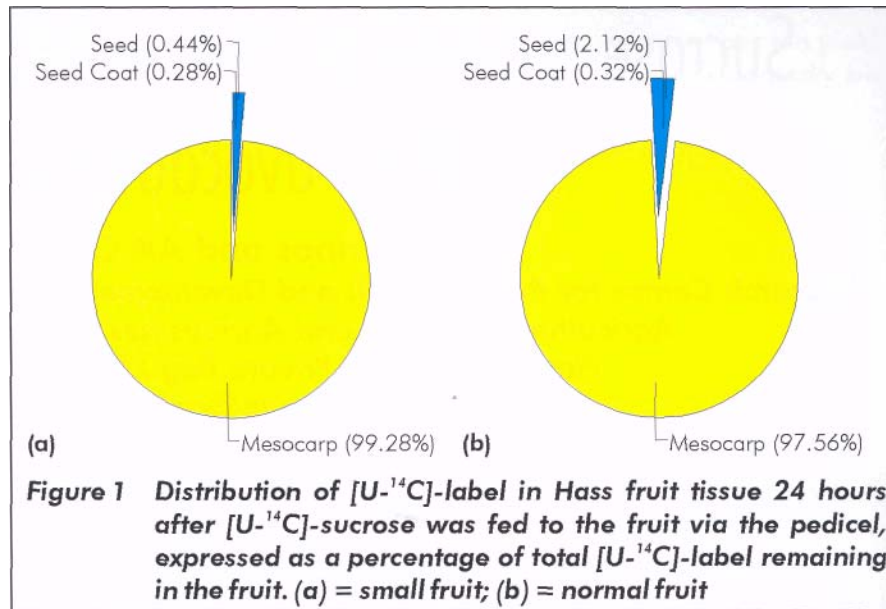
allocation between the normal and small fruit and if ABA plays any role in the expression of these different phenotypes,

SUCROSE MOVEMENT INTO THE FRUIT

In plants sucrose is the most abundant compound found in the phloem sap, and is the primary form (Sheen *et al.* 1999; Sturm & Tang, 1999) in which assimilated carbon is transported. In some species sugar alcohols (e.g. sorbitol, mannitol and in avocado this may include the abundant [Liu *et al.* 1996] sugar alcohol perseitol) and sugars of the raffinose series (e.g. raffinose and stachyose) are the principal forms of transported sugars, but where these predominate sucrose is always present. Sucrose serves as a compound for the long distance transport of metabolites; to drive osmotic solute movement; activate/repress many genes (Koch, 1996); and via the sucrose sensing pathway can modulate transport activity and assimilate partitioning (Chiou & Bush, 1998). It is understood that the availability, supply and composition of photoassimilates, such as sucrose (as well as plant hormones, minerals and water) to the fruit play a crucial role in fruit development and hence affect fruit size. In Hass the availability of these does not appear to be limiting as small and normal fruit occur side by side with no pattern with respect to distribution (Cowan, 1997), and thus the ability of the fruit to compete for these factors seems to be of primary importance.

Solute allocation is the distribution of assimilates from the site of synthesis and/or storage to the sites of utilization, as determined by the ability of the sink to attract solutes. The ability of the fruit to compete for and import (Herbers & Sonnewald, 1998) solutes (such as sucrose) is termed the sink strength. Sink strength thus determines the amounts of solutes allocated to the sink and it is affected by sink respiration; sucrose metabolism (Ho, 1996) (especially sucrose cleavage); solute/sugar partitioning (within the cellular compartments); transpiration; and the conductivity of the solute pathway.

Feeding of small and normal fruit with [U-¹⁴C]-labelled sucrose (Figure 1) showed that sucrose moves into the seed coat of normal fruit and from there into the seed.

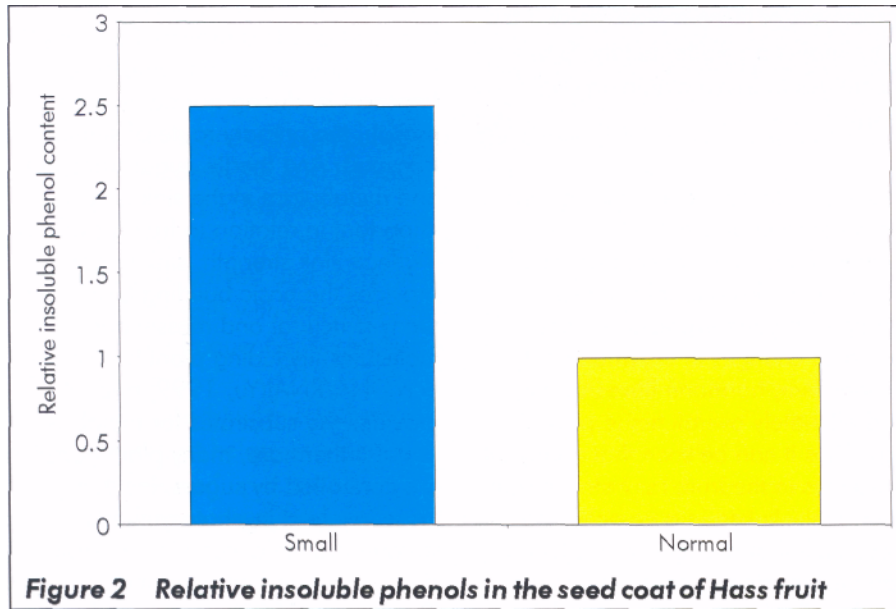


However in small fruit sucrose movement into the seed coat is very much reduced. As this sucrose does not enter the seed coat, it is not unloaded from the phloem and hence appears to accumulate in the mesocarp, instead of entering the seed and mesocarp, as it would in normal fruit. These results suggest two things. Firstly, that there is reduced phloem unloading and/or sucrose transport in the seed coat of the small fruit, which under normal circumstances is considered to be the primary site of phloem unloading in avocado fruit tissue. Secondly, relative to the normal fruit the small seed is neither getting any sucrose nor exerting significant sink strength. Whether this is the cause or effect of small fruit remains to be elucidated.

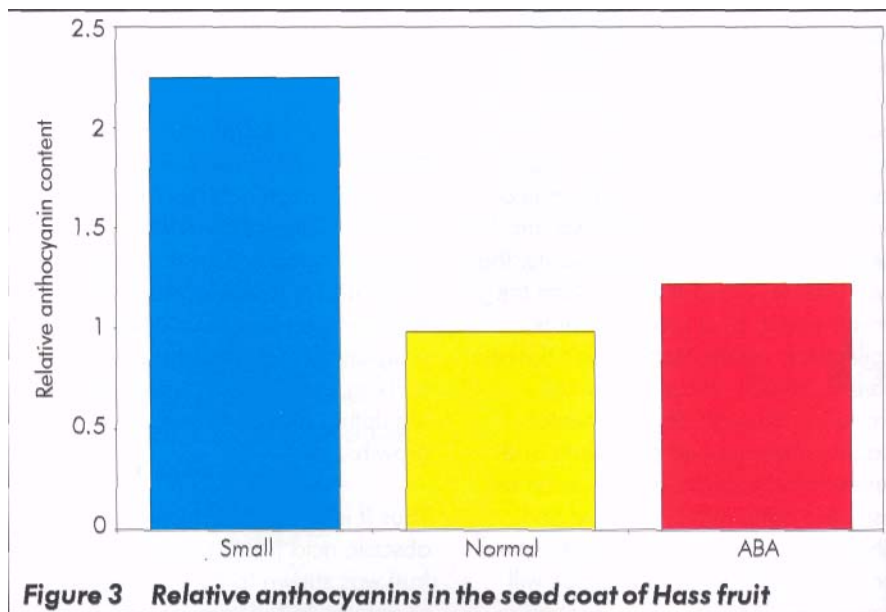
Collectively, transport through the seed coat tissue and the rest of the fruit tissue will be influenced by three factors: structural inhibition of solute movement; a change in plasmodesmatal conductivity; and a change in sink metabolic activity.

SUGAR IMPORT AND SEED COAT ULTRASTRUCTURE

Ultra-structural studies show that the small seed coat is highly degenerate, with structure-less deposits occurring in some cells, completely occluding them. The small seed coat is also highly reduced in comparison to the normal seed coat and shows extensive lignification, a phenomenon that is often associated with a loss of cell wall extensibility, cell growth and cell-to-cell communication (Boudet, 1998). Furthermore observation with UV light microscopy and electron microscopy suggest the small seed coat has a lot of phenolic-like compounds deposited both within the cell and on the outer edges of the seed coat. Quantification of insoluble phenols, by partial hydrolysis with concentrated sulphuric acid, suggests that insoluble phenols occur in much higher concentrations in the seed coat of small fruit (Figure 2).



Like lignin, the deposition of these amorphous unreactive compounds can be expected to limit solute movement between adjacent cells. Interestingly the seed coat of small and ABA treated fruits had elevated anthocyanin levels (Figure 3).



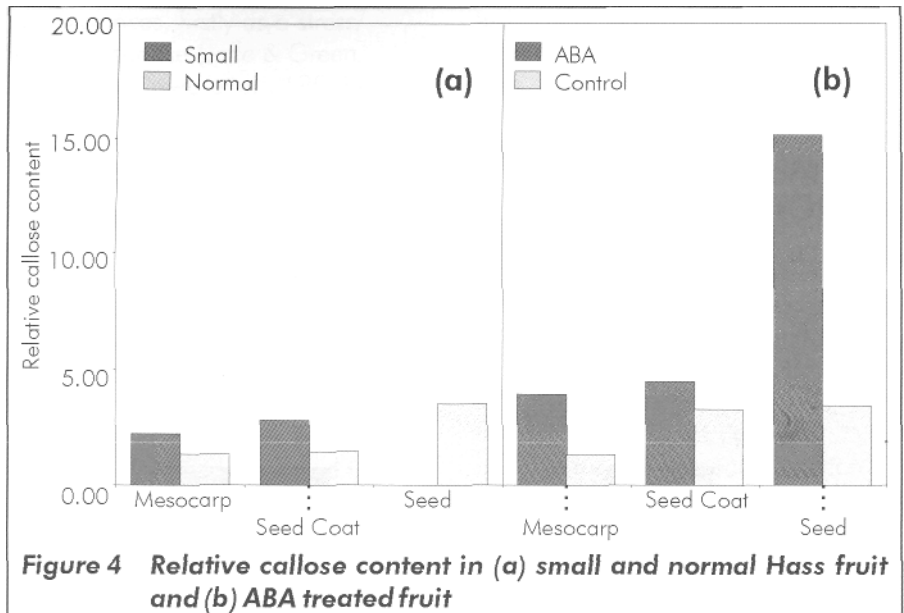
These strongly pigmented compounds will contribute to the colour of the small seed coat. Anthocyanins are known to scavenge/quench free radicals (Phippen & Simon, 1998; Park, 1999) and be synthesised in response to some stresses, such as light, pathogen-related elicitors or nutrient deficiency (Noh & Spalding, 1998). Free radicals are often produced in excess when the plant is subject to stress, such as high light

intensities, temperature extremes, mineral deficiency, drought and salinity stress and pathogen infection. This suggests there is another possible link of the small fruit phenotype to stress and the so-called 'stress hormone' ABA, which is known to act as a negative growth regulator and play an important role in many developmental and physiological processes throughout the life cycle of the plant (Davies, 1995). Oxidative damage has also been suggested to induce localized necrosis (Kawasaki *et al.* 1999) and secondary wall differentiation (Potikha *et al.* 1999), similar to that seen in the small seed coat.

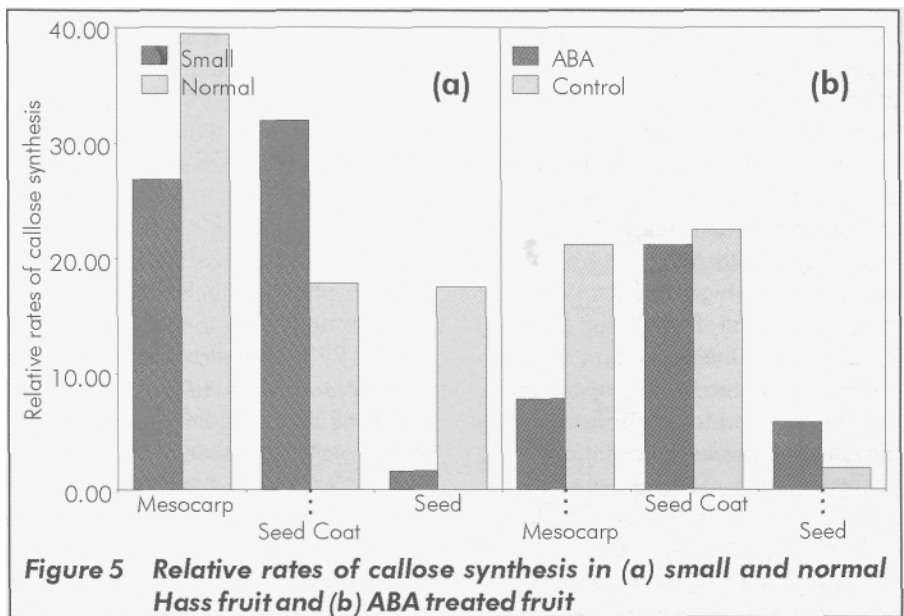
The highly vascularised seed coat supplies photo-assimilates, mineral nutrients and water to the seed (Steyn *et al.* 1993) and allows for communication between the developing embryo within the seed and the mesocarp and parent plant (Blumenfeld & Gazit, 1971). We have also established it to be the primary route of sucrose unloading. Casual observation indicates that the small fruit phenotype is always associated with early seed coat senescence and/or death and it is apparent that seed coat senescence/death can occur at any stage of fruit development, resulting in the cessation of rapid growth (Cowan *et al.* 1997). Interestingly this drying-up of the seed coat is also characteristic of mature normal fruit, and is believed to accompany the cessation of embryo growth and herald the accumulation of lipids in the mesocarp (Blumenfeld, 1970). Thus a loss of seed coat conductivity, both symplastically and apoplastically, due to seed coat degeneration, will limit solute allocation to the seed and embryo, and from these tissues to the mesocarp. It is believed that the seed, and especially the embryo, plays an integral role in controlling fruit development and in maintaining sink strength, and the isolation of these tissues will obviously affect overall fruit physiology.

SYMPLASTIC CONTINUITY: PLASMODESMATAL CONDUCTIVITY IN SMALL AND NORMAL FRUIT

The second area where solute allocation may be affected is in transport through the plasmodesmata. Plasmodesmata are small pores that link adjacent cells and allow for relatively high rates of symplastic transport to occur between neighbouring cells. Transport through these channels can be affected in several ways. The channel proteins may interact (Ehlers & Kollmann, 1996); the annulus may be occluded by the deposition of some substance; the extra cellular ring may contract to reduce the pore size; or the supporting structures in the cell wall may disintegrate causing the plasmodesmata to collapse. Callose, a structural polysaccharide frequently deposited in response to wounding or stress, is one such substance that may block (Gerber *et al.* 1998) or constrict the plasmodesmata. Staining with aniline blue showed that callose is localized to the plasmodesmata in avocado fruit tissue. We found callose occurred in larger amounts in the mesocarp and seed coat of the small fruit (Figure 4a), but was lower in the seed.



The rates of callose synthesis followed a similar pattern (Figure 5a).



Callose is synthesized under two circumstances, firstly as a stress/wound response (Jaffe & Green, 1988; Skalamera *et al.* 1997) and secondly during cell plate formation during cell division (Vaughn *et al.* 1996). Thus as the small seed has ceased to grow, but is still metabolically very active, it can be expected to have very low rates of callose synthesis and levels of callose. Whilst the high levels of callose in the seed coat and mesocarp of the small fruit, which do not display as active cell division as in the normal fruit, suggests that this substance has been deposited in response to something,

possibly stress. To test the hypothesis that callose is deposited in response to stress we treated fruit with ABA, and found indeed callose content is higher in ABA treated fruits (Figure 4b). However callose synthesis was lower in ABA treated fruits (Figure 5b) suggesting that ABA does not enhance callose synthesis but rather reduces callose turnover. Interestingly incubation of seed coat tissue in various sugar solutions too showed no significant differences in callose synthesis activity.

Turner and co-workers (1994) showed that callose is localized in the wall around the plasmodesmata in maize root tips and suggested that such a configuration would allow callose deposition to subtly constrict the collar and so restrict plasmodesmatal conductivity. Thus the elevated callose levels in the mesocarp and seed coat of the small fruit may contribute to a loss of symplastic continuity. Similarly the elevated callose levels in ABA treated fruit too may be responsible for the observations of Moore-Gordon *et al.* (1998).

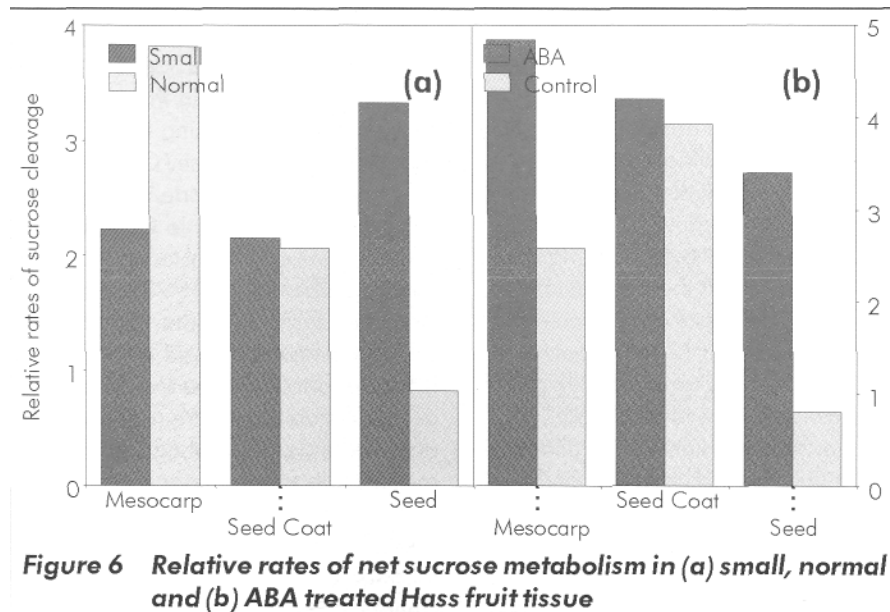
Previous work done on avocado suggested the occurrence of some granular material blocking the annulus of the plasmodesmata in ABA treated fruit (Moore-Gordon, 1997). These were electron dense and as callose is electron lucent are assumed to be of some other matter, such as protein. To see if this was protein the plasma membrane and endoplasmic reticulum, which spans the plasmodesmata, were isolated and the proteins separated. To date, however, no unique proteins have been observed in small fruit. These, however, may have been leached during isolation and this aspect of plasmodesmatal control is still under investigation.

SUGAR METABOLISM AND ACCUMULATION IN FRUIT TISSUE

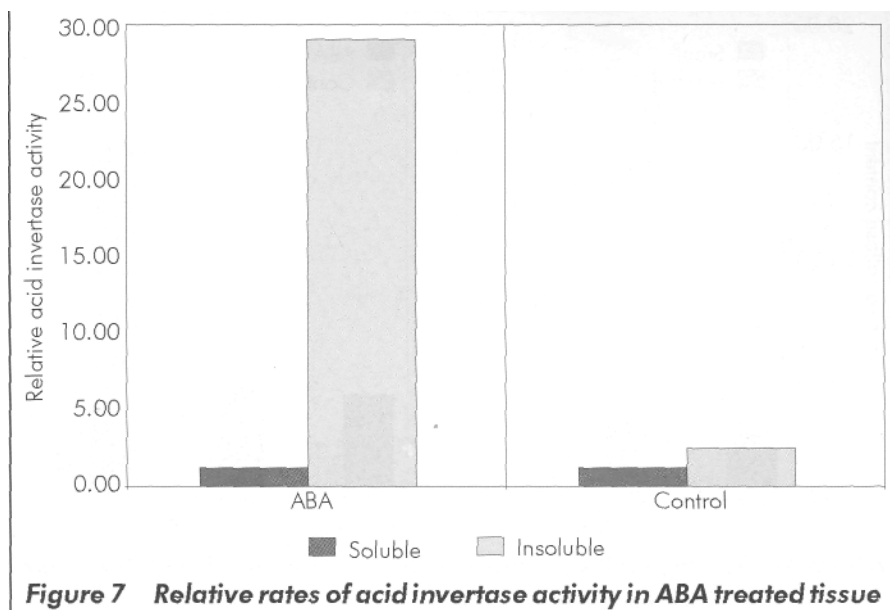
This brings us to the third way in which solute allocation may be controlled. Solute allocation is also controlled by the attractive strength of the sink, tissues as determined by sugar metabolism and compartmentation, which allows for a sugar gradient to be created between the parent plant and the fruit and so ensure continued sugar uptake (Stitt, 1996). This is largely affected by sucrose metabolism, content/composition and respiration.

In young developing fruit we can expect high enzyme activity in the symplast i.e. inside the cell. As the fruit develops, and it accumulates oil and sugar, there is a shift towards metabolism in the extra cellular region, i.e. in the cell wall. The enzymes, sucrose synthase (in both the cleavage and synthesis directions), acid invertase (both insoluble and soluble forms) and sucrose phosphate synthase were assayed. Of the sugar metabolising enzymes examined, the insoluble form of acid invertase shows the most striking difference in activity in small and normal fruit. Acid invertase cleaves sucrose to fructose and glucose, the insoluble form being important in the control of cell division, plant development and sugar partitioning between the source and sink (Sturm & Tang, 1999). Acid invertase is high in the mesocarp of normal fruit, and high in the seed coat, and seed of small fruit, whilst low in the seed of normal fruit. By combining all the enzyme activities results, it becomes apparent that net sucrose metabolism follows a similar trend (Figure 6a), with the small fruit having low rates of sucrose metabolism in the mesocarp and high in the seed, and vice versa for the normal fruit. The overall picture obtained is that the mesocarp of the small fruit shows enzyme activity that is

similar to that associated with the conclusion of fruit development.



ABA treatment seems to modulate sugar metabolism, especially that of insoluble acid invertase, to induce a response that too favours apoplastic metabolism (Figure 6b). This observation is clearly supported by the reduction in soluble acid invertase activity and the elevation of insoluble acid invertase activity associated with tissue incubated with ABA (Figure 7).



The net result of this change in sugar metabolism will be a change in soluble sugars, especially glucose, in the fruit tissue. The trend seen in small fruit typifies that of fruit in the later stages of development, and is in part mimicked by ABA treatment. Accompanying this is a reduction in the accumulation of starch in the seed of small fruit and an increase in respiration rates in small fruit. Suggesting two things. Firstly that respiration rates have been elevated to try and maintain sink strength, which has been shown to be correlated to high respiration rates (Nakano *et al.* 1998). Secondly, the small fruit is struggling to meet its metabolic needs and that starch that has been stored during fruit development to aid seed germination is now being directed to the maintenance of the small fruit seed. Sugar starvation within the seed will impact on the pattern of gene expression resulting in substantial changes in physiological and biochemical processes, such as starch breakdown (Journet *et al.* 1986) with the goal of sustaining respiration and essential metabolic processes (Yu, 1999), and so negatively impact on growth.

CONCLUSION

It is apparent that small avocado fruit is associated with reduced sucrose transport to the seed and mesocarp due to a combination of:

1. Physical constraints arising from the degeneration, lignification and phenolic deposition in the seed coat, the region from which imported sugars are distributed.
2. Reduced symplastic continuity associated with loss of plasmodesmata and a reduction in plasmodesmatal conductivity mediated by callose deposition and possible protein interaction within the micro channel.
3. A change in sucrose metabolism, favouring apoplastic sucrose metabolism, and in conjunction with increased respiration and reduced sugar accumulation in the fruit.

ABA treatment seems to be associated with many of these responses, though in many respects indirectly. And evidently this effect of ABA shows a clear interaction with some of these potential sites of solute allocation control. Whether elevated ABA is a cause or consequence of altered sugar transport, metabolism and accumulation is unclear. However we suggest elevated ABA, possibly in response to localized stress (as it is unlikely a stress experienced by the whole tree will only affect certain fruits, unless they are at a crucial stage of development when subjected to that stress) or some other signal, alters sucrose metabolism, favouring that of the apoplast. The change in sugars, especially glucose, in the extra cellular space can be expected to subsequently impact on cell differentiation, fruit development and sugar partitioning between the sink and the source. Accompanying this change in sugar content and ABA levels will be a change in callose turnover so reducing plasmodesmatal conductivity and so reducing transport through these micro channels. A reduction in transport, especially of simple sugars, small proteins and some nucleic acids, through the plasmodesmata and from the seed coat will subsequently affect gene expression, metabolism, the cell cycle, growth and development ... which can be visually manifested as a small fruit.

ACKNOWLEDGEMENTS

The authors would like to thank THRIP, SAAGA and the University of Natal for financial assistance. Everdon Estates and Rusty Roodt are acknowledged for access to their orchards and their contribution of fruit.

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